REMARKS

The Claim Amendments

Claims 1, 3, 13-21, 30, and 33 have been amended and claims 2, 4-12, 22-29, and 31-32, and 34-36 have been previously canceled. New claims 37-39 have been added. Applicant has amended claim 1 so that it is drawn to a chemically modified nucleic acid molecule having the following features: (1) it comprises a sense strand and a separate antisense strand, each strand having one or more pyrimidine nucleotides and one or more purine nucleotides; (2) each strand of the nucleic acid molecule is independently 18 to 27 nucleotides in length; (3) the antisense strand of the nucleic acid molecule comprises 18 to 27 nucleotides that are complementary to a PGF-1 RNA comprising SEQ ID NO: 257; (4) the sense strand of the nucleic acid molecule is complementary to the antisense strand, and comprises an 18 to 27 nucleotide portion of the PGF-1 RNA sequence; (5) about 50 to 100 percent of the nucleotides in the sense strand and about 50 to 100 percent of the nucleotides in the antisense strand of the nucleic acid molecule are chemically modified with modifications independently selected from the group consisting of 2'-O-methyl, 2'-deoxy-2'-fluoro, 2'-deoxy, phosphorothioate and deoxyabasic modifications; and (6) one or more of the purine nucleotides present in one or both strands of the nucleic acid molecule are 2'-O-methyl purine nucleotides and one or more of the pyrimidine nucleotides present in one or both strands of the nucleic acid molecule are 2'-deoxy-2'-fluoro pyrimidine nucleotides.

Amended claim 1 is fully supported by the specification as filed, for example, inter alia, at pages 6, 7, 8, 9, 10, 12-13, 14, 15, 16, 17, 18-20, 21, 22, 28-30, 31, 37-40, 139, and 141. Support is also found in the priority documents, for example, U.S. Provisional patent application No. 60/363,124 (see, pages 4, 5, 8, 9, 10, 11, 12, and 392).

In addition, claims 13, 14, 15, 18, 19, and 20 have been amended to clarify that 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more of the specified purine or pyrimidine nucleotides has the specified modification. Support for the amendment is found in the specification at, for example, pages 28-30. Support is also found in the priority documents. See, e.g., U.S. Provisional patent application No. 60/363,124 (see, pages 9-11).

In addition, the claims have been further amended merely to correct dependencies and other matters of form.

Claim 30 has been amended to recite that the terminal phosphate on the antisense strand is at the 5'-end. Support for the amendment can be found in the specification at, for example, pages 20 and 21, as well as in U.S. Provisional patent application No. 60/431,105 (pages 8 and 9).

New claim 37 depends from claim 1 and recites a nucleic acid molecule that is chemically modified with one or more phosphorothioate internucleotide linkage, 2'-O-methyl ribonucleotide, 2'-deoxy-2'-fluoro ribonucleotide, 2'-deoxy ribonucleotide, universal base nucleotide, 5-C-methyl nucleotide, inverted deoxyabasic or any combination thereof. Support for new claim 37 can be found in the specification at, for example, pages 12-40. Support is also found in the priority documents. See*, e.g.,* U.S. Provisional patent application Nos. 60/363,124 (*see*, pages 8-12).

New claim 38 depends from claim 19 and recites a nucleic acid molecule wherein additionally 1, 2, or 3 purine nucleotides in the sense strand are 2'-O-methyl nucleotides. Support for new claim 38 is found in the specification at, for example, pages 28-30. Support is also found in the priority documents. See*, e.g.,* U.S. Provisional patent application Nos. 60/363,124 (*see*, pages 9-11).

New claim 39 depends from claim 1 and recites a method of inhibiting the expression of PGF-1 comprising administering the nucleic acid molecule of claim 1 to a human subject in need thereof under conditions that allow for inhibition of PGF-1 expression. Support for new claim 39 is found in the specification at, for example, pages 7, 11, and 46-51, as well as in U.S. Provisional patent application No. 60/363,124 (*see*, pages 15-17).

Amendments to the claims are made without prejudice and do not constitute amendments to overcome any prior art or other statutory rejections and are fully supported by the specification as filed. Additionally, these amendments are not an admission regarding the patentability of subject matter of the canceled or amended claims and should not be so construed. Applicant reserves the right to pursue the subject matter of the previously filed claims in this or in any other appropriate patent application. The amendments add no new matter and applicants respectfully request their entry.

A complete listing of all the claims, in compliance with the revised amendment format, is shown above.

35 USC §103 Rejections

Claims 1, 3, 13-20, 30, 35, and 36 have been rejected under 35 USC §103(a) as being obvious over Carmeliet et al (WO 01/85796 A2), in view of GenBank Accession Number NM_002632, Hammond (2001); Elbashir (EMBO J, 20:6877-6888 (2001)), and Parrish et al (Molecular Cell, Vol. 6, 1077-1087, 2000). Applicant respectfully traverses the rejection.

The Office relies on Carmeliet et al to teach the use of antisense and ribozymes targeted PIGF mRNA. However, the Office acknowledges that Carmeliet does not disclose the nucleotide sequence encoding human PGF-1 comprising SEQ ID NO: 257. The Office relies on GenBank Accession Number NM_002632 for its teaching of the sequence of PGF-1 comprising SEQ ID NO: 257.

The Office relies on Hammond for its teaching that RNAi possesses characteristics that are superior to antisense; however, the Office recognizes that Hammond does not teach the use of siRNA. The Office relies on Elbashir for its teaching of siRNA having 21-23 nucleotides and alleges that Elbashir teaches "that siRNAs containing nucleotide modifications known in the art such as 2'-O-methyl substitutions can stimulate RNA interference." (Office Action, page 5). The Office relies on Parrish for allegedly teaching that some nucleotide modifications, including 2'-fluoro modifications, are tolerated in interfering RNAs.

The Office argues that it would have been obvious to combine the teachings of the cited references to make double stranded RNAs that are optionally modified siRNAs capable of down-regulating the PGF-1 gene because Carmeliet teaches the use of nucleic acid based inhibitors to antagonize the activity of PGF-1, Hammond teaches the superiority of dsRNA over antisense, Elbashir provides motivation to make siRNA because it teaches that siRNA are efficient mediators of RNAi, and Parrish provides motivation to use chemically modified siRNA because it teaches that such modifications are tolerated in RNAi.

Furthermore, the Office argues that one would have a reasonable expectation of success in making siRNA to down-regulate the expression of PGF-1 gene because Carmeliet teach that PGF-1 gene expression can be inhibited by the activity of RNA inhibitors, and Hammond, Elbashir, and Parrish teach the feasibility of designing a dsRNA molecule to inhibit the activity of a target gene having a known nucleotide sequence. The Office argues further that one would have a reasonable expectation of success in making siRNAs having sugar, base, and backbone modifications because Elbashir and Parrish teach that interfering RNAs containing such modifications are active in provoking RNAi

Under 35 U.S.C. § 103(a), to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the references, when combined must teach or suggest all the claim limitations. *See* MPEP §2143.

None of these references, alone or in combination, make obvious the presently claimed double stranded nucleic acid constructs because the cited references do not teach or suggest all of the claim elements. None of the references teach or suggest a siRNA molecule in which about 50 to 100 percent of the nucleotide positions in **both** strands of the nucleic acid molecule are chemically modified with 2'-O-methyl, 2'-deoxy-2'-fluoro, 2'-deoxy, phosphorothioate and/or deoxyabasic modifications. Furthermore, none of the references teach or suggest a siRNA molecule in which one or more of the purine nucleotides present in one or both strands are 2'-O-methyl purine nucleotides and one or more of the pyrimidine nucleotides present in one or both strands are 2'-deoxy-2'-fluoro pyrimidine nucleotides.

Neither GenBank Accession Number NM_002632, nor Carmeliet teach or suggest a chemically modified siRNA molecule. Elbashir is the only prior art reference that mentions chemical modification of short interfering RNA. However, the Office mischaracterizes Elbashir in stating that Elbashir teaches "that siRNAs containing nucleotide modifications known in the art such as 2'-O-methyl substitutions can stimulate RNA interference." Elbashir does not provide any examples of siRNA having 2-O-methyl modification that stimulate RNAi. In fact, the only example of siRNA having 2-O-methyl modification is one in which the siRNA has 100% 2-O-methyl modifications and has no RNAi activity. Thus, not only does Elbashir fail to teach selective incorporation of 2'-O-methyl guanine and 2'-O-methyl adenine residues into siRNA, but it expressly teaches away from the use of highly modified siRNA constructs, in particular, 2'-deoxy and 2'-O-methyl modified constructs. Specifically, Elbashir teaches that extensive substitution with 2'-deoxy or 2'-O-methyl modifications abolishes RNAi (see Figure 4 and pages 6881-6882). Further, given that Elbashir fails to mention 2'-deoxy-2'-fluoro modification, it certainly fails to teach 2'-deoxy-2'-fluoro pyrimidine nucleotides.

The Office also relies on Parrish; however, Parrish does not teach chemically synthesized double stranded siRNA molecules comprising 2'-deoxy-2'-fluoro modifications, much less 2'-deoxy-2'-fluoro pyrimidine modifications. Instead, Parrish teaches long dsRNA molecules, not chemically synthesized short interfering RNA molecules, as recited in the instant claims. All of the 2'-deoxy-2'-fluoro modifications taught by Parrish were introduced by enzymatic incorporation of 2'-deoxy-2'-fluoro uridine nucleotides into long double stranded RNA molecules (>700 nt), **not** selective synthetic incorporation of 2'-deoxy-2'-fluoro uridine and 2'-deoxy-2'-fluoro cytidine nucleotides (collectively

2'-deoxy-2'-fluro pyrimidine nucleotides) into short interfering RNA molecules. Furthermore, Parrish fails to teach or suggest a siRNA molecule wherein about 50 to 100% of both strands are chemically modified and wherein one or more of the purine nucleotides present in one or both strands of the nucleic acid molecule are 2'-O-methyl purine nucleotides and one or more of the pyrimidine nucleotides present in one or both strands of the nucleic acid molecule are 2'-deoxy-2'-fluoro pyrimidine nucleotides. In fact, Parrish expressly teaches that modifications of the antisense strand decrease RNAi activity (see pages 1081 and 1082, Figures 5 and 6). Thus, Parrish expressly teaches away from highly modified siRNA constructs, such as the instantly claimed constructs.

None of the cited references, alone or in combination, teach the specific pattern of chemical modification recited in the claims; that is, 2'-O-methyl purine nucleotides in one or both strands and 2'-deoxy-2'-fluoro pyrimidine nucleotides in one or both strands of the nucleic acid molecule, where about 50-100% of each strand are chemically modified with 2'-O-methyl, 2'-deoxy-2'-fluoro, 2'-deoxy, phosphorothioate and/or deoxyabasic modifications. Furthermore, a double stranded nucleic acid molecule having a specific pattern of chemical modification is not a structure that would be rendered obvious by a general knowledge in the art of chemical modification of other oligonucleotides, such as ribozymes and antisense.

Despite the lack of teaching in the cited references, the Office suggests that it would have been obvious to make double stranded nucleic acid molecules having chemical modifications with a reasonable expectation of success because Elbashir and Parrish teach that interfering RNAs having sugar, base, and backbone modifications are active in provoking RNAi. The Applicant strongly disagrees.

None of the art at the time of filing provides any insight into whether highly modified double-stranded siRNA nucleic acid constructs, such as the claimed siRNA constructs, would function. Indeed, Elbashir expressly teach away from highly modified siRNA constructs. Elbashir teaches that extensive substitution with 2'-deoxy or 2'-O-methyl modifications abolishes RNAi activity. In a section tellingly entitled, "The siRNA User Guide," Elbashir expressly teaches away from highly modified siRNA constructs:

"The siRNA User Guide"

> Efficiently silencing siRNA duplexes are composed of 21 nt sense and 21 nt antisense siRNAs and **must** be selected to form a 19 bp double helix with 2 nt 3'-overhanging ends. 2'-deoxy substitutions of the 2 nt 2'-overhanging ribonucleotides do not affect RNAi, but help to reduce the costs of RNA synthesis and may enhance RNAse resistance of siRNA duplexes. **More extensive 2'-deoxy or 2'-O-methyl**

> **modifications reduce the ability of siRNAs to mediate RNAi**, probably by interfering with protein association for siRNAp assembly. (emphasis added).

(see, Elbashir et al., page 6885; see also Tuschl et al., U.S. Publ. No. 2002/0086356, paragraphs [0178] to [0179]).

Because the only teaching in the cited art addressing the issue of the degree of modifications tolerated in siRNA molecules expressly states that more than a few end modifications should be avoided, it could not have been obvious to make the highly modified constructs now being claimed with a reasonable expectation of success.

Furthermore, in the time period of about 2000-2001 the high potency of siRNAs and the relative stability of the double stranded structure (as compared to antisense and ribozymes) tended to suggest that no additional chemical modification of the molecules would be necessary. It was common knowledge to those skilled in the art at the time of the invention that single stranded RNA and DNA is much more susceptible to nuclease attack than double stranded nucleic acids. Thus, it was thought the relatively unstructured antisense and ribozyme nucleic acid molecules would be expected to require additional stabilization while the substantially double-stranded siRNA molecules would not. An example of this thinking is seen in Elbashir I (EMBO Journal, 20:6877-6888 (2001)) and Tuschl (U.S. Publ. No. 2002/0086356), where an emphasis was placed on modifying the 3' single stranded ends of the siRNA, with little effort made to modify the double stranded 5' ends. *See,* p. 6881, "*2'-deoxy- and 2'-O-methyl-modified siRNA duplexes*;" p. 6884, "*Sequence effects and 2'-deoxy substitutions in the 3' overhang.*"

The methods paper of Elbashir II (Methods 26:199-213 (2002)) best exemplifies the mindset of the day, that additional chemical modifications are unnecessary for effective RNAi activity. This paper gives specific instructions for designing and carrying out an RNAi experiment. On page 202, Protocol 1 (step 2) states that:

> Independent of the selection procedure described in Fig. 2, synthesize the sense siRNA as 50-(N19)TT, and the sequence of the antisense siRNA as 50-(N'19)TT, where N'19 denotes the reverse complement sequence of N19. N19 and N'19 indicate ribonucleotides; T indicates 2'-deoxythymidine.

Thus, RNA duplexes with dTdT 3' ends were considered the correct substrate for carrying out RNAi experiments. The terminal TT was there primarily to make chemical synthesis easier and less expensive, although some minor protection from single-stranded ribonucleases was also considered a possibility (Elbashir I, Elbashir II). Finally, Elbashir II makes specific mention of four suppliers of siRNA duplexes for RNAi research; all four companies supply the reagents in the standard form described in Protocol 1 of Elbashir II.

As stated above, there was no motivation to seek chemically modified siRNAs during the period in question. Elbashir I is the only paper from the period that describes a significant attempt to modify siRNAs away from their own standard of RNA with TT overhanging ends.

In the section entitled "*2'-deoxy- and 2'-O-methyl-modified siRNA duplexes*" (see pages 6881-6882), Elbashir describes the effect of chemical modification on the activity of the siRNA duplex to mediate RNAi. The authors state:

> To assess the importance of the siRNA ribose residues for RNAi, duplexes with 21 nt siRNAs and 2 nt 3'-overhangs with 2'-deoxy- or 2'-O-methyl-modified strands were examined (Figure 4). Substitution of the 2 nt 3'-overhangs by 2'-deoxynucleotides had no effect and even the replacement of two additional ribonucleotides by 2'-deoxynucleotides adjacent to the overhangs in the paired region produced significantly active siRNAs. Thus, 8 out of 42 nt of a siRNA duplex were replaced by DNA residues without loss of activity.

Thus, while 2'-deoxy substitutions at the 3'-terminal positions were permitted, there was no mention of any active siRNAs using 2'-O-methyl modifications, even at the terminal positions. Furthermore, because complete substitution of one or both siRNA strands with either 2'-deoxy or 2'-O-methyl residues resulted in a complete loss of RNAi activity, (as demonstrated in both Tuschl and Elbashir I) the results of Elbashir I suggests that modification of internal nucleotides positions reduced the ability of siRNAs to mediate RNAi, probably by interfering with protein interactions or siRNP assembly.

It was not until much later in 2003 that reports began appearing in the scientific literature regarding the use of chemical modifications other than 3'-terminal 2'-deoxy substitutions in siRNAs. *See, e.g.,* Chiu and Rana, 2003, RNA, 9:1034-1048; Allerson *et al.,* 2005, *J. Med. Chem.* 48, 901. It is readily apparent from the publication record that those working in the RNAi field initially followed the teachings of Elbashir, outlined above, in designing siRNAs for experimental work. Only more recently has the use of chemical modifications become generally accepted.

Therefore, no motivation existed at the time of the invention to cleave PGF-1 RNA using siRNA molecules chemically modified with 2'-O-methyl purine and 2'-fluoro pyrimidine modifications. In fact, because the *only* teaching in the cited art addressing the issue of the degree of modifications tolerated in siRNA molecules expressly states that more than a few end modifications should be avoided, it could not have been obvious to make the highly modified constructs now being claimed with a reasonable expectation of success. The present claims go directly against the express teachings of the art. Thus, due to the teachings of Elbashir (and Tuschl), there was no reasonable expectation of success in using extensively chemically modified siRNA molecules with, *e.g.,* 2'-O-methyl and 2'-fluoro modifications.

Central to its obviousness rejection is the Office's assertion that the recited elements, in particular the chemical modifications, were independently known in the art (drawing from the pool of siRNA, dsRNA, antisense, and ribozyme art). For reasons previously discussed, Applicant strongly disagrees with this assertion. However, even if, for the sake of argument, the recited elements were known in the art, the Supreme Court has recently cautioned that claims composed of several elements are not proved obvious merely by demonstrating that each element was, independently, known in the prior art. *KSR International, Co. v. Teleflex, Inc*, (U.S. 2007) at p.14.

Instead, the KSR Court instructs that an obviousness analysis requires a further consideration of several factors, known as the Deere factors.

> "[T]he scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved. Against this background the obviousness or nonobviousness of the subject matter is determined. Such secondary considerations as commercial success, long felt but unresolved needs, failure of others, etc. might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented." (emphasis added) *KSR v. Teleflex*, citing *Graham v. John Deere Co. of Kansas City*, 383 U.S. 1, 17-18 (1966).

In this case, the level of ordinary skill in 2002 and early 2003 was relatively low with respect to chemically modified siRNA. At the time of filing, the ordinary artisan did not have insight into whether highly modified double-stranded siRNA nucleic acid constructs, such as the claimed siRNA constructs, would function.

In fact, the state of the art at the time of the claimed invention emphasized the high potency and enhanced stability of dsRNA compared to single stranded nucleic acid, such as antisense. Thus, it was thought that extensive chemical modification of siRNA would not be necessary. Further, studies at the time actually demonstrated that extensively modified double-stranded siRNA nucleic acid constructs would not function. The only siRNA art at the time of filing, i.e., that of Elbashir and Tuschl, demonstrated that highly modified siRNA did not retain RNAi activity. These results were further confirmed by the teachings of Parrish, which showed that long, double stranded RNA also lost its activity when extensively chemically modified. Thus, regardless of the non-analogous antisense and ribozyme art, the relevant siRNA art taught away from extensively modifying siRNA.

Importantly, the Supreme Court in *KSR v. Teleflex* reconfirmed an earlier Supreme Court holding that when the prior art teaches away from combining certain known elements, discovery of a successful means of combining them is more than likely to be nonobvious. *KSR v. Teleflex*, citing *United States v. Adams*, 383 U.S. 39, 40 (1966). In particular, the KSR Court pointed out that a determination of obviousness should not require a person skilled in art to ignore known facts. *Id.* In this case, the Office errs in dismissing the fact that the relevant siRNA art teaches away from extensive chemical modification of siRNA.

In analyzing the final "Deere" factor, Applicant notes that there are several important differences between the prior art and the instant claims. Unlike the teachings in the prior art references, which teach siRNA having minimal chemical modification at the ends of the molecule, the instantly claimed double stranded nucleic acid molecules have about 50%-100% chemical modifications on each strand and also have one or more 2-O-methyl purines and one or more 2'-deoxy-2"-fluoro pyrimidines on either strand.

In addition, the *Graham v. John Deere* case instructs one to further consider secondary factors, such as the failure of others, to dislodge the determination that a claim is obvious. In this case, the Applicant has succeeded in discovering a pattern of chemical modifications that allows extensively modified siRNA to retain its RNAi activity, while others, such as Elbashir, Tuschl and Parrish, have failed. The success of the Applicants in view of the contemporaneous failure of others indicates the non-obviousness of the claimed invention.

Finally, based on its reasoning that most, if not all, inventions rely upon building blocks long since uncovered, and claimed discoveries almost of necessity are combinations of what is already known, the Supreme Court concluded that it is important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does. Thus, the Supreme Court mandates the identification of convincing, articulated reasoning with rational underpinning to support the legal conclusion of obviousness. *KSR v. Teleflex* at 14.

In view of the prior art teachings discussed above, and in particular the art that teaches away from extensive modification of siRNA, Applicant submits that the Office has failed to identify a convincing reason for a person of ordinary skill in the art to combine the chemical modification art with the siRNA art to arrive at an extensively modified double stranded nucleic acid molecule targeted to PGF-1 RNA having the claimed pattern of chemical modification.

For the reasons discussed above, the cited references, alone or in combination, do not render the instantly claimed chemically modified double stranded nucleic acid molecules obvious. Accordingly, Applicant respectfully requests withdrawal of the 35 USC §103 Rejection.

Obviousness-Type Double Patenting Rejection

Claims 1, 3, 13-21, 30, and 33 are provisionally rejected on the grounds of nonstatutory obviousness-type double patenting as allegedly being unpatentable over claims 1-35 of copending Application No. 10/922,761. Without acceding to the merits of the Office's allegation, and merely to expedite prosecution, Applicant will consider filing a terminal disclaimer over Application No. 10/922,761 at the appropriate time.

Conclusion

In view of the foregoing amendments and remarks, the applicant submits that the claims are in condition for allowance, which is respectfully solicited. If the examiner believes a teleconference will advance prosecution, she is encouraged to contact the undersigned as indicated below.

Respectfully submitted,

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